Protocol for a Routine Vent PCR

Overview

All components should be mixed and spun down prior to pipetting. These recommendations serve as a starting poin; in order to maximize amplification the reaction conditions may require optimization (see Vent DNA Polymerase Guidelines for PCR Optimization Protocol).

Buffers

ThermoPol Reaction Buffer (NEB# B9004)
Diluent D (NEB# B8004)

Protocol

1. Prepare the following 50 µl reation in a 0.5 ml PCR tube on ice:

COMPONENT	VOLUME (µl)	FINAL CONCENTRATION
ThermoPol Reaction Buffer (10X)	5 µl	1X
Deoxynucleotide (dNTP) Solution Mix (10 mM)	1 µl	200 µM
Upstream Primer (10 µM stock)	0.5-2.5 µL	0.1-0.5 µM
Downstream Primer (10 µM stock)	0.5-2.5 µl	0.1-0.5 µM
DNA Template	determined by user	
Vent DNA Polymerase*	0.25-0.5 µl	0.5-1 unit
$MgSO_4$	(optional)	1-6 mM
Nuclease-free water	Bring reaction to a final volume of 50 µl	

**Due to the difficutlies in pipetting small volumes of enzyme, Vent DNA Polymerase can be diluted in Diluent D (NEB# B8004S) or 1X reaction buffer. For example, 1 µl of Vent DNA Polymerase is mixed with 4 µl of diluent and 1 µl of that mixture is added to the reaction. Enzyme diluted in Diluent D can be stored at -20°C for future use.*

2. Gently mix the reaction and spin down in microcentrifuge.
   If the thermocycler does not have a heated cover, add one drop of mineral oil to the reaction tube to prevent evaporation.
3. Cycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	2-5 minutes
20-30 Cycles	95°C 55-65°C 72°C	15-30 seconds 15-30 seconds 1 minute per kb
Final Extension	72°C	5 minutes
Hold	4-10°C	